University of North Carolina, Chapel Hill
Committee on the Protection of the Rights of Human Subjects (Medical IRB)

APPLICATION FOR APPROVAL OF RESEARCH INVOLVING HUMAN SUBJECTS

DATE: April 25, 2007 **IRB STUDY NUMBER (leave blank if new submission)**: 03-1139

TITLE OF STUDY: Inflammatory Changes in Asthmatics Exposed to Concentrated Chapel Hill Coarse Air Particles

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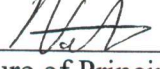
Study Staff: Martha Almond RRT; Margaret Herbst RN MSN; Carole Robinette MS; Hazel Shepherd RN MSN; Lynne Newlin-Clapp; Sally Ivins BA; Maryann Bassett rn; Debbie Levin RN; and Tracey Montilla RN.
NAME AND PHONE NUMBER OF RESEARCH COORDINATOR, IF APPLICABLE: Peg Herbst, RN, MSN, 966-2879

NAME OF FUNDING SOURCE: United States Environmental Protection Agency

I. Agreements

Principal Investigator:
I certify that each of the above-named co-investigators has accepted his/her role in this study. I agree to a continuing exchange of information with the Committee on the Protection of the Rights of Human Subjects (IRB). I agree to obtain IRB approval before making any changes or additions to the project. I will provide progress reports at least annually, or as requested. I agree to report promptly to the IRB all unanticipated problems or serious adverse events involving risk to human subjects. A copy of the consent form will be given to each subject and the signed original will be retained in my files. If the study involves treatment of UNC Hospitals patients, a copy of the consent form will be placed in each subject's medical record.



Signature of Principal Investigator — Date: 04/30/07

Signature of Faculty Advisor if P.I. trainee or Non-Faculty — Date

Department Chair of P.I. (or Vice-Chair if Chair is investigator or otherwise unable to review):
I have reviewed this research study. I believe the research is sound, that the study design and methods are adequate to achieve the study goals, and that there are appropriate resources (financial and otherwise) available to the investigator. I support it, and hereby submit it for further review.

Signature of Department Chair — Department — Date

II. Summary Checklist

ARE THE FOLLOWING INVOLVED?	**YES**	**NO**
Surveys, questionnaires or interviews *If research is limited to use of surveys, questionnaires or interviews, Submit **Exemption Application Form** instead of this application.*	☐	☒
Existing Patient Records and/or Specimens *If research is limited to study of existing medical records and /or samples, Submit **Short Form** instead of this application.*	☐	☒
Investigational Drug(s) **IND#** *If "yes", do you intend to use the UNC Hospitals Investigational Drug Service?*	☐ ☐	☒ ☐
Approved drugs for "non-FDA-approved" conditions	☐	☒
Placebo(s)	☐	☒
Experimental devices, instruments, machines **IDE#**	☐	☒
Genetic studies on subjects' specimens	☒	☐
Storage of subjects' specimens for future, as-yet-undesignated research *If "yes", see **Instructions for Submitting IRB Applications for Research that Includes the Storage of Human Biologic Specimens**.*	☒	☐
Fetal tissue	☐	☒
Videotaping, audiotaping, filming of subjects	☐	☒
Non-patient volunteers	☒	☐
Patients as subjects	☐	☒
Minors (less that 18 years old) *If "yes", indicate:* **Age range to years**	☐	☒
Do you intend to target your enrollment at: -Students or staff as subjects?	☐	☒
-Non-English-speaking subjects?	☐	☒
-Decisionally impaired or mentally incompetent subjects?	☐	☒
-Prisoners, parolees and other convicted offenders as subjects?	☐	☒
-Pregnant subjects?	☐	☒
Will HIV tests be performed?	☐	☒
Will subjects be studied at off-campus sites?	☐	☒
Is this a multicenter study? *If "yes", is UNC-CH the sponsor or coordinating center?*	☐ ☐	☒ ☐
Diagnostic or therapeutic ionizing radiation, or radioactive isotopes, which subjects would not receive otherwise *If "yes", approval by the Radiation Safety Committee is required.*	☐	☒
Recombinant DNA or gene transfer to human subjects *If "yes", approval by the Biologic Safety Committee is required.*	☐	☒
Is this an oncology study? *If "yes", submit this application directly to the Oncology Protocol Review Committee.*	☐	☒
Will subjects be studied in the General Clinical Research Center? *If "yes", obtain GCRC Addendum from the GCRC and submit complete application (IRB application and Addendum) to the GCRC.*	☐	☒

III. Required Education in Human Subjects Protection

UNC policy requires that all persons engaged in research involving human subjects must complete training in ethical conduct of research and protection of subjects. This applies to all research, regardless of funding source. For further information, including what options are acceptable in fulfillment of these requirements, see http://www.med.unc.edu/irb/Education2.htm

Individuals who have completed training should have been entered into the Human Subjects Training Database maintained by the Office of Research Services (ORS). To print documentation, visit http://zeppo.admin.unc.edu/isapi/certweb.dll and enter the names of each individual involved with this research project. Names not returned by the database are not recognized as having satisfied the education requirement. For questions regarding the database, please contact ORS at 962-7757.

WITH THIS APPLICATION, please submit the printout from the ORS database, verifying that each individual involved in the research (including faculty, staff, students and outside collaborators, if responsible to this IRB) has satisfied the education requirements.

IV. Potential Conflict of Interest

The following questions apply to any investigators or study staff involved with industry-sponsored research, and/or their immediate family members (spouse, dependent children, others). Within the past 12 months or the next 12 months, have you or will you:

Receive any form of personal compensation from the Sponsor, including salary, consulting fees, honoraria, royalties, equipment, etc.?	☐ YES	☒ NO
If so, does or will that compensation exceed $10,000?	☐ YES	☒ NO
Have an ownership interest of any nature in the Sponsor or product under study, including equity, stock options, etc.?	☐ YES	☒ NO
If so, does or will that interest exceed $10,000 in value?	☐ YES	☒ NO
If so, does that interest represent more than 5% ownership in the Sponsor?	☐ YES	☒ NO
Hold any position with the Sponsor, including officer, director, trustee, consultant, member of advisory board, etc.?	☐ YES	☒ NO
Have an intellectual property interest on any technology or invention used in this study, including patent rights, copyright, etc.?	☐ YES	☒ NO
Have a conflict of interest disclosed through the University's annual evaluation policy that relates to this research study?	☐ YES	☒ NO

If the answer is "YES" to any of the questions above, please include an explanation with this application. As with any changes to the research itself, relationships or interests that develop later should be brought to the IRB's attention for further consideration.

V. Description of Proposed Research Activity

Entire application should not usually exceed 5 single-spaced pages using a 12-point font.

1. **Purpose and Rationale:** Provide a brief summary of the background information, state the research question(s), and tell why the study is needed. Avoid an extensive literature review.

More than 100 different epidemiology studies report that inhalation of air pollution particles less than 10 microns in aerodynamic diameter (PM_{10}) is associated with increased mortality and morbidity. Those most at risk appear to be elderly individuals with pre-existing cardiopulmonary disease. These associations are stronger for "fine" particles (less than 2.5 microns) than for "coarse" particles (between 2.5 and 10 microns, PM_{10}). Although epidemiology studies fail to find any significant association between coarse PM and mortality, the association between coarse PM and **disease morbidity** remains a significant concern to the EPA. Consequently, the EPA office charged with recommending PM standards has concluded that one of their highest priorities is a better understanding of whether coarse particles cause adverse health effects in humans.

Animal toxicology studies and human *in vitro* studies suggest that coarse PM may cause pro-inflammatory effects. For example, coarse particles collected from the city of Zerbst (Germany) and instilled into mice caused significant release of IL-8 into the blood; fine particles collected from the same city did not. A study in Fresno CA by Pinkerton et al. exposed mice to concentrated coarse PM and found this caused lung inflammation. Likewise, Harkema et al (2003, Toxicological Sciences) demonstrated that rats showed persistent alveolitis and Type II cell proliferation after exposure to coarse PM (carbon black particles). Hetland et al (2003, Toxicological Sciences) recently demonstrated that coarse PM compared to fine and ultra-fine PM, was more toxic and induced a similar or higher potency pro-inflammatory cytokine response (IL-8, IL-6) in the human alveolar cell line A549. Researchers at the EPA Human Studies Division (Clincial Research Branch) at UNC Chapel Hill have used a newly developed impactor capable of collecting large quantities of size-fractionated PM. In their studies, exposure of cultured human primary airway epithelial cells to these fractions revealed that coarse PM elicited a comparable release of several pro-inflammatory mediators (IL-8, GM-CSF, PGE_2) compared to fine PM and that these responses were blunted by adding Polymixin B, an inhibitor of endotoxin. Instillation of these particles into the lungs of animals also resulted in significant findings.

There is ample evidence to suggest that asthmatics may be more susceptible to the adverse health effects of PM exposure, and the notion that asthmatics may be more susceptible to coarse PM than healthy non-asthmatics is a valid one. For example, coarse PM has biogenic components (e.g. pollen fragments, endotoxin, bacterial cell walls) that are known exacerbants of asthma. Individuals with asthma may be preferentially affected by coarse PM exposure, due to the deposition pattern of PM_{10} in the lung. Coarse PM_{10} compared to fine particles has been shown to primarily deposit in the central airways, the primary site of asthma pathology, so it is likely that these particles will affect disease outcome. Moreover, individuals with asthma have as a feature of their disease, elevated airways inflammation and potentially impaired innate host defense capabilities. These two factors may contribute to asthmatics having increased susceptibility to PM_{10} exposure.

A limitation of the *in vitro* and *in vivo* instillation studies described above is the uncertainty associated with extraction of particles from filters or other substrates. It is not clear if all components have been extracted or if the extraction process alters the chemistry of the particles. Furthermore, particles tend to agglomerate during extraction and their altered size range results in potential deposition in the lung at sites different from where "real world" unextracted particles would deposit when inhaled. Thus it is important to confirm the *in vitro* and animal instillation studies by controlled human inhalation studies using "real world" particles whenever possible. The recent acquisition by the EPA HSD of a particle size fractionator will permit us to directly measure the response of asthmatics to concentrated coarse PM exposure. An identical study in healthy non-asthmatics using this device is currently underway at USEPA HSD.

The purpose of this study is to expose subjects with mild to moderate asthma to concentrated coarse PM and compare the results with those currently being obtained in a parallel study conducted by the EPA on

healthy human volunteers. Comparable levels of coarse PM will be used in this study that are being used in the PM study on healthy subjects.

Our **hypothesis** is that coarse PM will cause lung inflammation and injury and further decrease innate host defense capability in asthmatic volunteers, perhaps to a greater degree to that observed following exposure of healthy young subjects to coarse PM. However, because coarse particles are deposited preferentially in the larger airways, we expect that the portion of the BAL that samples large airways (bronchial fraction) will have more evidence of inflammation than the portion that samples the distal airways. We do not expect to see decreased heart rate variability in these subjects since it was not observed in a previous EPA CAPs study with asthmatic subjects (only in the study of elderly subjects) and therefore we expect a similar outcome with coarse particles. We expect that the results of this study will clearly show that coarse PM is capable of causing deleterious pulmonary effects in asthmatics. This study and similar studies are needed to allow the EPA to determine if coarse PM causes sufficient health effects to susceptible populations to warrant continued regulation of these particles.

2. **Subjects:** Specify number, age, gender, ethnicity, and whether healthy volunteers or patients. If patients, specify the disease or condition and indicate how potential subjects will be identified. If pregnant women are excluded, or if women who become pregnant are withdrawn, specific justification must be provided. NIH applications require that women, minorities, and children be included or that their exclusion be justified. If children are involved, refer to "Children as Research Subjects".

Approximately 15 asthmatic individuals with mild to moderate disease severity, aged between 18 and 40, will serve as volunteers. Not all subjects who sign consent and enroll actually finish the study due to scheduling conflicts, relocation etc. For this reason, up to 25 subjects will be enrolled until 15 subjects have completed the study. There are no gender or racial restrictions. However, pregnant women or nursing mothers will be excluded from participation since possible effects of coarse particles on a fetus or young infant are unclear. All female subjects will be tested for pregnancy at the time of admission into the study and again immediately prior to exposure.

3. **Inclusion/Exclusion criteria:** List required characteristics of potential subjects, and those that preclude enrollment.

Inclusion criteria:

1. Positive history of asthma (wheezing, chest tightness, and reversible airway obstruction)
2. Baseline $FEV_1/FVC = OR > 60\%$;
3. Positive history of bronchial hyperreactivity to methacholine defined as a minimum of 20% FEV_1 decrement (above that of diluent alone) after inhaling < 5.0 mg/ml aerosol of methacholine
4. Withhold antihistamines for 48 hrs prior to testing
5. Withhold inhaled bronchodilator 6 hrs prior to exposure.

Exclusion Criteria:

1. Use of oral steroid therapy within the past month
2. Physician directed emergency treatment for asthma
3. Exacerbation of asthma within the preceding 6 months
4. Current smoker or smoking history within 1 year of study (defined as more than one pack of cigarettes in the past year); greater than 5 pack-years during lifetime.
5. Oxygen saturation below 97% at the time of physical exam.
6. Any chronic medical condition including active pulmonary disease, cardiovascular disease, neurological disease, liver disease, kidney disease, muscular disease, diabetes, other endocrine disease, hematologic/lymphatic disease, immune deficiency or autoimmune disease.
7. Hepatitis B carriers
8. Any significant risk factors for cardiovascular disease (e.g. blood pressure > 140/90).
9. Active cancer, history of cancer within the last 5 years, untreated cancer.
10. No exposure will be conducted within 6 weeks of a respiratory tract infection.

Allowances:

1. Use daily theophylline therapy
2. Use daily inhaled steroids
3. Use inhaled cromolyn
4. No history of respiratory diseases other than allergic rhinitis and asthma.

Atopic asthmatic subjects will not be studied during active allergy season. In addition, individuals unable to discontinue substances that could potentially alter their inflammatory response to PM (e.g. NSAIDs, antioxidants) for at least 2 days prior to exposure will not be allowed to participate. The following procedure will be followed in the event that any exclusion criteria item becomes known or positive during the course of study participation:

- Subject participation in the study will stop
- The study physician will be notified immediately of the occurrence in question and will, based on his/her discretion, decide on the appropriate of course of action for the subject (i.e. continue in the study, post-pone participation until further notice, seek further attention)

4. **Full description of the study design, methods and procedures:** Include the type of experimental design; study procedures; sequential description of what will be asked of/done to subjects; assignment of subjects to various arms of the study if applicable; doses, frequency and route of administration of medication and other treatment if applicable; kinds of data to be collected; primary outcome measurements; and follow-up procedures. If the study involves treatment, distinguish standard care procedures from those that are research. If the study is a clinical trial involving patients as subjects and use of placebo control is involved, provide justification for the use of placebo controls. This section (4) should generally not exceed 2 single-spaced pages using 12-point type.

EXPERIMENTAL DESIGN

This will be a double-blind study in which each subject will be randomly exposed to filtered air and air containing concentrated coarse particles (diameter > 2.5 microns) in an exposure chamber. The study will follow a repeated measures design with subjects serving as their own control. There will be a minimum of 4 weeks between an individual subject's exposures.

Subject Qualification

Screening: Patients will be recruited by the Westat Corporation (see section 12 below). During an initial telephone interview, the volunteers will receive information regarding the study and their eligibility status will be assessed. Volunteers whose responses indicate that they are likely to meet the criteria will be scheduled for an appointment in the Westat recruitment office in the USEPA Human Studies Facility (HSF). At that time the study protocol will be outlined, a screening informed consent form will be signed, and a medical history form completed which contains information on general personal and family medical history.

Physical exam: Subjects who are not excluded during the initial screen will be scheduled for a physical examination in the HSF by a licensed physician or nurse practitioner. During this visit subjects will sign an informed consent for a physical and the medical history form completed during screening will be discussed. Subjects will then undergo an abbreviated physical exam including blood sampling (SMA-20 serum chemistry and a complete blood count with differential), 12 lead ECG to screen for baseline cardiac arrhythmias and ST segment and T wave abnormalities, and pulse oximetry.

Bronchoscopy physical exam: A physical exam will also be performed by a physician certified in pulmonary medicine to ensure the subjects are suitable candidates for bronchoscopy. Prior to the examination the subject will be asked to sign a bronchoscopy physical exam informed consent. This examination will include a more thorough assessment of the subject's nares and throat. A spirometry test and/or chest X-ray may be done if indicated by the history and physical.

Training session: Those subjects who are not excluded on the basis of the physical exam will undergo a training session to familiarize them with the study protocol, obtain informed consent to complete the study, and to answer any questions they might have regarding their participation in the study. During this visit the subject's first exposure session will also be scheduled.

Exposure Day 1

Pre-exposure: On the day of the study, the subject will report to the medical station in the HSF at which time the general health of the subject will be evaluated and the appropriate pre-exposure measurements (HRV, pulmonary function, blood sampling, urine pregnancy test for females) will be completed. Electrodes for telemetry and HRV measurement will be placed. The skin in the areas of electrode placement will be cleaned and shaved (if necessary) to ensure that the electrodes will remain securely attached. For HRV measurement, a standard 3-channel, 5-lead configuration will be used in which leads will be placed approximately in the areas above the manubrium of the sternum, at midsternum, and above the xiphoid process and on the right and left mid-axillary line. These leads will be connected to a Holter monitor (Zymed) and will remain in place for approximately 24 hours. Standard telemetry leads will also be placed and removed when the patient leaves for the day. The subject will then be allowed to relax for 30 minutes in a reclined position after which a 10-minute resting HRV measurement will be obtained. Blood samples will then be collected and lung function will be measured following the HRV measurements. The subject will then enter the exposure chamber for filtered air or particle exposure.

Exposure: In the chamber, the subject will sit on a cycle ergometer and will undergo a schedule of exercise consisting of 15 minutes of exercise (exercise intensity = 65 erg) followed by 15 minutes of rest. This cycle will be repeated 4 times during the 2-hour exposure. Telemetry will be monitored continuously throughout the exposure. Subjects will also be monitored continuously by trained personnel for signs of respiratory distress, chest pain, significant cardiac arrhythmias, ataxia, or other signs of distress. The subjects will be aware that they may terminate their exposure at any point should they deem necessary. Concentrated particles will be generated from ambient Chapel Hill air drawn from above the roof of the HSF and subsequently concentrated approximately 25 fold. Ambient air gases and particles smaller than 2.5um will enter the chamber but not be concentrated. PM-concentrations will be measured continuously. The actual concentration of particles that the subjects will be exposed to will be dependent on the particle concentration of Chapel Hill air on the day of the study. Therefore, the particle concentration in the chamber may vary from day to day. Concentrations of PM less than 10 microns in Chapel Hill vary somewhat seasonally but usually average between 15-30 $\mu g/m^3$. Concentrations of PM between 2.5 and 10 microns usually account for about 20% of that mass, or about 3-6 $\mu g/m^3$. If these particles are concentrated about 25 fold, we anticipate the average exposure to coarse PM will be between 75-150 $\mu g/m^3$. This is comparable to levels of fine CAPs that we have exposed volunteers to for the past 3 years. Because of the daily variability in PM levels, it is likely that on some days CAPs levels will exceed the anticipated average. On those days with high levels of outdoor PM, subjects will not be exposed to more than 400 $\mu g/m^3$ of coarse CAPs; either clean dilution air will be added to keep PM concentrations below this value or the exposure will be terminated. Healthy young subjects have previously been exposed to similar levels of fine PM in our facility with no adverse effects and we do not expect coarse PM to be significantly more potent than fine PM. Since the exposures are somewhat dependent on the outdoor concentrations of particles, there may be some days in which there are too few particles outdoors for an exposure (e.g. if it is raining). If this happens when a subject is scheduled to be exposed to particles, the subject will be rescheduled and entitled to receive compensation ($12/hr) for the time spent at the EPA facility that day.

Post-exposure: Immediately following the exposure, the subject will be allowed to rest for 20 minutes and then HRV measurements, blood sampling, and lung function measurements will be taken as described above. Subjects will then be given instructions regarding the holter monitor, NPO status, and the time they are to return to the HSF the next day.

Study procedure day 2

The morning after day 1, the study subjects will return to the HSF to undergo a brief medical evaluation and HRV measurement. The subject will then be allowed to relax for 30 minutes in a reclined position after which a 10-minute resting HRV measurement will be obtained and a blood draw will be taken. The Holter monitor will then be removed and the subject will be prepared then undergo bronchoscopy.

Exposure day 2 regimen will be repeated no less than 4 weeks later, at which time the subject will be exposed to coarse CAPs or clean air, whichever was not used in the prior exposure. Thus individuals will act as their own controls.

OUTCOMES:

Lung Function will be measured before and after exposure. Subjects will perform spirometry, and single breath diffusing capacity (DLCO) on a Sensor Medic Vmax pulmonary function system according to the standard algorithm published by the American Thoracic Society.

Bronchoscopy. Subjects will undergo fiberoptic bronchoscopy with BAL and protected brushings to recover small numbers of airway epithelial cells approximately 24 hours after exposure. BAL measurements will include, but not be limited to, differential cell counts and soluble markers of lung injury and inflammation (e.g. cytokines, prostaglandins, LDH, fibronectin). Epithelial cells removed by protected brushings will be analyzed for microbiology changes in expression of inflammatory genes and other genes indicative of pulmonary injury or response to PM.

Bronchoscopy with BAL and cytology brushes will be performed by a licensed physician board certified in pulmonary medicine and experienced in the use of a fiberoptic bronchoscope and will be assisted by 2 experienced R.N.s. The subject will be monitored during the procedure using telemetry, pulse oximetry, and continuous blood pressure monitoring. The subject's nose and pharynx will then be anesthetized. The subject will gargle approximately 5cc of 4% lidocaine and 2% liquid lidocaine will be dripped into each nostril. This will be followed by insertion of 4% lidocaine jelly into each nostril. The level of anesthesia will be monitored by placing 2 sterile cotton swabs into one of the subject's nostrils. Should the subject feel any discomfort, more lidocaine jelly will be used. The procedure will begin after the subject's nose and oropharynx are adequately anesthetized.

Prior to beginning the procedure, a towel will be placed over the subject's eyes to shield against spraying liquids and a nasal cannula providing oxygen (2L/min) will be placed on the side of the nose not used to pass the bronchoscope. A flexible fiberoptic bronchoscope will be passed into the subject's nose and oropharynx and the position will be monitored visually by the physician on a television screen with the use of a camera interfaced to the bronchoscope. Upon nearing the vocal cords, a 1-2% solution of lidocaine will be sprayed through a channel in the bronchoscope to anesthetize them. The bronchoscope will then be passed into the trachea. An injection of 1-2% lidocaine will be made at the main carina and in the proximal right and left bronchi to anesthetize the airway and to minimize the subject's cough reflex during the remainder of the procedure.

BAL will be performed on the side opposite to the side that the brush biopsy will be taken. The bronchoscope will be wedged in a subsegmental bronchus. A volume of up to approximately 270cc of sterile saline (one 20cc and up to five 50cc aliquots) will be injected and immediately aspirated through the channel of the bronchoscope. The lavage fluid aliquots will be collected in polypropylene tubes and kept on ice until processed. After the BAL is complete, the bronchoscope will be repositioned for the bronchial brushes. A sterile cytology brush connected to a long wire will be passed through the channel of the bronchoscope and will be used to obtain surface airway epithelial cells. The brush procedure will be carried out by rubbing the epithelial mucosal surface. After each brushing, the brush is removed and the recovered cells are dislodged from the brush by stirring in a test tube containing sterile tissue culture medium. No more than 6 brushes will be done, each in a different site of the mainstream bronchus. The physician will monitor the sites for hemostasis before removing the bronchoscope from the airway. A 1:10,000 or 1:20,000 dilution of epinephrine will be available for injection through the bronchoscope if the bleeding is considered excessive.

The subject will be monitored for at least 1 hour by a nurse following the procedure. Chest electrodes will be removed if the ECG tracing is normal, the oxygen cannula will be removed if the oxygen saturation is satisfactory, and the Hep-lock will be removed if IV access is no longer needed. As a precaution, vital signs will be monitored at least every 30 minutes during recovery. The subject will be discharged by the physician if there are no signs of complications, if the subject has normal vital signs, has a gag reflex, and is able to tolerate oral intake without aspirating. Post-bronchoscopy spirometry may be performed at the discretion of the study

MD. Each subject will be given a pager number and telephone numbers for the medical station and the physician should follow-up be necessary. In addition, subjects will be contacted within 24-48 hours following discharge by a member of the nursing staff.

Heart Rate Variability (HRV) data will be gathered for 24 hours using a Zymed holter monitor. Specific 10 minute epochs to be analyzed for frequency domain variables include times immediately prior to exposure, immediately following exposure, and approximately 24 hours after exposure. Both time and frequency domain variables will be analyzed, as will abnormal responses (e.g. PACs, PVCs, bradycardia, tachycardia).

Peripheral venous blood samples. The medical staff will draw approximately 70 mls of blood from each volunteer before exposure, immediately after exposure, and 18 hours after exposure, for a total volume of about 210 mls over a 20 hour period. A small portion of the pre-exposure blood draw (2.7ml) will be used for genetic testing.

Samples will be placed into heparinized or citrate-coated tubes and refrigerated until the blood can be processed. Endpoint measurements will include, but not be limited to, the following: markers for specific and non-specific immune responses, coagulation factors, vasoactive factors, soluble components of PM (e.g. transition metals), and inflammatory genes of interest. Subjects will be asked to sign an additional consent form for ***Storing Biological Specimens <u>With</u> Identifying Information*** which will provide additional information regarding sample storage. During the course of this research, other researchers may request access to specimens (or data) for as-of-yet unspecified research that may or may not be related to the original research from which the specimens were derived. In these cases, provided appropriate UNC IRB approved consent has been obtained, these specimens (or data) will be provided without identifiers to these other researchers by employing a Data Use Agreement or Honest Broker model.

5. **Duration of entire study and duration of an individual subject's participation, including follow-up evaluation if applicable**: Include the number of required visits and approximate duration of each visit.

It is anticipated that the duration of this study will be approximately 18 months. Subject recruitment and screening is expected to be continuous throughout the study until the intended number of subjects is reached. Scheduling constraints imposed by concurrent studies in the Human Studies Division are expected to limit the rate at which subjects can be exposed to 1 per week. In addition to visits for screening and a qualifying medical examination (which should not exceed 2-3 hours total), each subject will visit the HSF on four occasions, grouped into two pairs. During the first visit, the subject will be exposed to air or coarse particles with a duration of about 3-4 hours. The next day the subject will return for bronchoscopy, with a duration of about 4 hours. This process will be repeated no sooner than 4 weeks later.

6. **Where will the subjects be studied?** If off UNC-CH campus, list locations.

All exposures will be carried out at the EPA Human Studies Facility on the UNC campus.

7. **Full description of risks and measures to minimize risks:** Include risk of psychosocial harm (e.g. emotional distress, embarrassment, breach of confidentiality, etc.) economic harm (e.g. loss of insurability) and legal jeopardy (e.g. disclosure of illegal activity) as well as known side effects of study medication, if applicable, and risk of pain and physical injury.

General measures to minimize the risks: Medical screening of the potential subjects is designed to exclude those that may be at risk from the study procedures. A physician is on call in the building whenever a subject is undergoing any procedure at the facility. The physician will terminate the procedure at any time if he feels that it would be injurious to the subject's well being to continue. Two nurses staff a fully stocked medical station and the University of North Carolina Hospital is two blocks from the HSF. On subsequent days after exposure and bronchoscopy, subjects will be urged to contact the medical station or the physician should they experience any of the following symptoms: epistaxis, persistent cough, chest pain, dyspnea, wheezing, hoarseness, or sore throat. Risks associated with specific study procedures are as follows:

- **Bronchoscopy with BAL and cytology brushes** may be associated with respiratory distress, bleeding, pneumothorax or even death. These risks are explained to the subject in full detail. More than 1200 bronchoscopy procedures have been performed without a serious incident at the Human Studies Division on the UNC-CH campus. Established protocols for bronchoalveolar lavage and brush biopsy ensure that the safety of the subject is given absolute priority. The subject's vital signs, oxygen saturation, and ECG are continuously monitored during the procedure and during recovery. Symptoms which may result in procedure termination include discomfort or anxiety, chest pain, ECG abnormality including tachy- or bradycardia, tachypnea, depressed respiration, moderate bronchospasm, moderate bleeding of the airways, epistaxis, arterial blood saturation less than 93% on 6L of supplemental oxygen, or significant changes in blood pressure.

  Discomfort of the nose and throat is a common risk of bronchoscopy and will be minimized through the use of lidocaine, which itself presents small risk to the subject. Lidocaine could potentially be absorbed through the nasal mucosa resulting in systemic effects such as bradycardia, hypotension, urticarial reactions, confusion, lightheadedness, euphoria, tremors, or seizures. To limit these effects, minimum necessary amounts of lidocaine jelly and liquid will be used. Because increased systemic absorption can occur through an inflamed nasal mucosa, subjects with a recent history of upper or lower respiratory tract infection will not be bronchoscoped. Subjects will be continuously monitored for signs and symptoms of lidocaine toxicity.

  Epistaxis is caused by trauma to the nose by the bronchoscope. This condition is minor and generally resolves on its own. Small streaks of blood in nasal secretions may be present for up to 12 hours following the procedure. If the bleeding becomes severe, the procedure will be terminated. The subject's anterior nasal passage will be packed with sterile gauze to stop the bleeding. If the bleeding fails to resolve with packing, the subject will be transferred to the UNC Hospitals Emergency Room.

  Bleeding in the lower airway may occur from trauma caused by the bronchoscope or brushing. The bleeding is typically minor and will typically spontaneously resolve in a matter of minutes. A 1:10,000 or 1:20,000 dilution of epinephrine will be available to stop the bleeding if does not spontaneously resolve. If epinephrine administration does not resolve the bleeding or if it is sufficiently severe to cause hemoptysis or hemoglobin desaturation, oxygen supplementation will be administered and the subject will be transferred to the UNC Hospitals Emergency Room. Epinepherine may be absorbed systemically through the mucosa resulting in transient headache, palpitations, and tachycardia. However, these effects are unlikely to occur due to the small doses used.

  There is a small risk of pneumothorax with brushing. Symptoms include dyspnea and chest pain. Subjects will be transferred to the UNC Hospitals Emergency Room if a pneumothorax is suspected.

  Low-grade fever (38-38.5°C) or pneumonia can occur in subjects undergoing bronchoscopy. The fever typically resolves within 18 hours without treatment or with acetaminophen. In previous studies at the HSF, <1% of subjects undergoing bronchoscopy have reported a fever after the procedure. Risk of pneumonia in the lobe involved in the procedure is also <1%. Symptoms of pneumonia could include fever, dyspnea, persistent or productive cough, and chest pain. The subjects will be asked to contact the physician or nurses at the medical station if symptoms persist or if they experience a fever higher than 38.5°C.

- **Pulmonary function tests** (spirometry) are standard non-invasive techniques that are commonly used in studies of pulmonary function on populations of all ages and entail little or no risk to the subject. The intrabreath technique uses acetylene uptake for Qc measurement. Large doses of acetylene are associated with nausea, vomiting, and headache. However, our subjects will be exposed to low concentrations of acetylene (0.3%) for a brief period of time (single inhalation and exhalation), thus we anticipate that the risks of these complications to our subjects will be quite low.
- **ECG and heart rate variability** are standard non-invasive techniques commonly used for heart rate and rhythm analysis and entail little or no risk to the subject. There is the possibility that preparation of the skin for electrode placement and removal may cause skin irritation, itching, or soreness in some subjects.
- **Blood sampling** risks, including pain and hematoma formation, are considered mild and minimal. A licensed RN will take blood samples.
- **Exercise testing** is associated with minimal risk in healthy individuals. It is possible, however, that a previously unidentified preexisting cardiac condition will be uncovered. To minimize this risk, a thorough medical screening will be performed prior to exercise and heart rate and rhythm will be monitored while the

subject is exercising. Other side effects of exercise, including occasional muscle soreness, cramps, and general fatigue, are considered temporary and not deemed harmful.

- **Particle exposure** will occur in mild to moderate asthmatics who are otherwise in good health without pre-existing cardiopulmonary disease. Based on current knowledge, a single exposure to coarse air particles will not have any permanent adverse health effects at the concentrations being used in this experiment. Heart rate, electrocardiogram, and pulse oximetry will be monitored continuously. Subjects will also be monitored for significant respiratory distress or dyspnea, chest pain, significant cardiac arrhythmias, pallor, and ataxia. Subjects will be aware that they can terminate their exposure for any reason and still receive compensation for the entire exposure session. The investigator or duty physician will end the exposure if the subject is found to be suffering from any adverse effect.

8. **Benefits to subjects and/or society:** The possibility of benefit to society should be clearly distinguished from the possibility of benefit to the individual subject, if any. If there is no direct benefit to the individual subject, say so. Do not list monetary payment as a benefit.

Subjects will receive no direct benefit from participating in this study other than receiving a medical examination including blood work, spirometry, and an ECG. Subjects will have full access to their records. For society, this study will provide new information on the effects of coarse air particles on regional lung function, inflammation, and the cardiovascular system. Epidemiology studies do not currently show a strong association between coarse PM and mortality/morbidity, and some have suggested that the current EPA standard on coarse PM may not be necessary. However, a small number of recent animal toxicology studies, using different end points than the epidemiology studies, indicate that coarse PM may induce pulmonary injury comparable to that of fine particles. The results from this human study will provide important data that assist the EPA in determining whether or not to retain the current standard on coarse PM.

9. **Inducements for participation:** If monetary, specify the amount and how this will be prorated if the subject withdraws (or is withdrawn) from the study prior to completing it.

Subjects will receive monetary compensation for their time (approximately $12/hour) and participation in the study. A subject who is unable to complete the study for voluntary reasons will receive full compensation for his/her participation to that point. Subjects who do not complete the study for involuntary reasons will be paid for their participation up to the point of termination. Payment will be made after each segment of the study, unless the subject specifies otherwise.

The following table details the expected compensation for completion of the entire study:

Pre-study Qualifications	
Recruitment Screening	$15
Physical Exam	$15
Bronchoscopy Physical Exam	$20
Training	$12
Exposure Sessions (2 for 5 hours each)	$216
(Check-in, pre-testing, exposure and post-testing)	
Next day follow-up (2x4h)	$96
Blood sampling (6)	$150
24-hour Holter (2)	$200
Bronchoscopy (2)	$700
(BAL, cytology brushes, biopsy, recovery)	
Bonus for Completion of the Study	$50
Total Compensation	**1474**

In addition, subjects traveling from Durham and Raleigh will be paid an additional $6 and $11 per round trip, respectively, and all parking costs will be paid. Since the exposures are somewhat dependent on the outdoor concentrations of particles, there may be some days in which there are too few particles outdoors for an exposure (e.g. if it is raining). If this happens on a day a subject is scheduled to be exposed to particles, the

subject will be rescheduled and entitled to receive compensation ($12/hr) for the time spent at the EPA facility that day.

10. **Costs to be borne by subjects:** Include clinic fees, diagnostic and laboratory studies, drugs, devices, transportation, all professional fees, etc. If there are no costs to subjects, indicate this.

All procedures and costs directly related to participation in this study will be free of charge to the subjects.

11. **Statistical analysis:** If this is a single-center clinical trial, provide evidence that the sample size is sufficient to achieve the study aims and tell how the data will be analyzed. If a multicenter trial, indicate where and by whom statistical analysis will be performed.

To test our primary hypothesis that coarse PM causes lung inflammation and injury we will measure a number of endpoints (e.g. PMN infiltration, cytokine expression, lung function); however the primary end point of interest is influx of PMNs into the lung. In addition, several markers of systemic response to PM will also be measured (e.g. HRV, change in blood coagulation factors) but treated as exploratory hypotheses since there is currently no data to indicate they are affected by coarse PM. Statistical data analyses will consist of paired Student's t-tests for parametric variables and rank sum tests for non-parametric variables.

12. **Methods of recruiting:** Tell how prospective subjects are contacted. If they are UNC Hospital patients, initial contact should be made by their treating physician, or by someone whom the patients know to have legitimate access to their medical records (for example, a clinical director). This may be accomplished by means of a letter from that individual to prospective subjects, requesting the patient's permission to be contacted by the investigator.

Subjects will be recruited for this study by the Westat Corporation, which has recruited subjects for studies at the HSF since 1998. The manner in which this will be done is identical to that of past EPA studies and specific recruitment procedures are described in the subject recruitment protocol on file with the UNC Committee for the Protection of the Rights of Human Subjects. The population targeted will be residents of the Triangle area. *Every effort will be made to recruit women and members of racial minority groups into this study.* Advertisements will be placed in local newspapers. Volunteers will be asked to call the recruitment office. During the telephone interview, the volunteers will receive information regarding the study and their eligibility status will be assessed. Volunteers whose responses indicate that they are likely to meet the criteria will be scheduled for an appointment in the Westat recruitment office in the Human Studies Facility. At that time the entire study protocol will be outlined, and a medical history form will be administered.

13. **How will informed consent be obtained?** Describe the process. When the consent of a legally authorized representative is substituted for consent of the adult subject, explain why this is necessary. If non-English-speaking subjects will be enrolled, a consent form should be prepared in their foreign language. Someone who is fluent in the subjects' language must be available to interpret.

Before being selected as subjects, all volunteers will be required to read and sign a **form(s)** asserting that they have read and understood the following: 1) Subject participation is strictly voluntary, 2) The purpose of the study, 3) The nature and extent of subject participation, 4) The subject's rights to withdraw at any time, 5) The subject's right to privacy, 6) The risks associated with participation, 7) The method and schedule of compensation, and 8) The limits of the University and PI's liability.

One of the PIs will briefly describe the study and answer any questions that each subject might have regarding his/her participation, the safety of the procedures, issues related to payment, etc. The PI will then review the contents of the consent form(s) before he and the subject sign it. Subjects will have the opportunity to ask questions at any time during the study by contacting one of the PIs and/or the medical staff. Subjects will be asked to sign a written informed consent form(s) after all of their questions and concerns have been addressed. One signed copy of the written informed consent will be given to the subject while the investigators will retain the original.